

### **Amendments to the Specification**

**At page 14, please rewrite Paragraph [56] as follows:**

[0056] Figure 2 illustrates sequence information of HR-N and HR-C regions of SARS coronavirus S protein. The sequence of HR-N is given in SEQ ID NO:3 and that of HR-C is given in amino acids 2-41 of SEQ ID NO:40.

**At page 15, please rewrite Paragraph [0065] as follows:**

[0065] Figure 11 illustrates variations of HR-N and HR-C peptide sequences. Amino acid sequences of HR-N, Ala, Lys and Arg substituted, Aib=B substituted and Dxg=Z substituted derivatives are set forth in SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52 and SEQ ID NO:53, respectively. Amino acid sequences of HR-C, Ala, Lys and Arg substituted, Aib=B substituted and Dxg=Z substituted derivatives are set forth in SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60 and SEQ ID NO:61, respectively.

**At page 15, please rewrite Paragraph [0066] as follows:**

[0066] Figure 12 illustrates covalently constrained HR-N and HR-C peptides. . Amino acid sequences of HR-N and the 1i,i+4 lactam bridge; 2.i.i+4 lactam bridge and 1i,i+7 lactam bridge constrained derivatives are set forth in SEQ ID NO:50, SEQ ID NO:54, SEQ ID NO:55 and SEQ ID NO:56, respectively. Amino acid sequences of HR-C and the N and the 1i,i+4 lactam bridge; 2.i.i+4 lactam bridge and 1i,i+7 lactam bridge constrained derivatives are set forth in SEQ ID NO:58, SEQ ID NO:62, SEQ ID NO:63 and SEQ ID NO:64, respectively.

**At page 15, please rewrite Paragraph [0067] as follows:**

[0067] Figure 13 illustrates modulation of the "a" and "d" residue positions. Amino acid sequences of HR-N (916-950)HR-C (1151-1185) and Ile substituted into the hydrophobic core) derivative are set forth in SEQ ID NO:58, SEQ ID NO:59 and SEQ ID NO:65, respectively.

**At page 15, please rewrite Paragraph [0071] as follows:**

[0071] Figure 17 illustrates helix template immunogens and preparation methods. In Panel B, the HR-N (920-945) peptide and the HR-C(1161-1186) sequences correspondence to SEQ ID NO:110 and SEQ ID NO:111, respectively.

**At page 15, please rewrite Paragraph [0072] as follows:**

[0072] Figure 18 illustrates HR-N peptide sequences, HR-N1 to HR-N17. The nucleotide sequences encoding following peptides are set forth in the Sequence Listing as follows: encoding HR-N1, SEQ ID NO:5; HR-N2, SEQ ID NO:7; HR-N3, SEQ ID NO:9; HR-N4, SEQ ID NO:11; HR-N5, SEQ ID NO:13; HR-N6, SEQ ID NO:15; HR-N7, SEQ ID NO:17; HR-N8, SEQ ID NO:19; HR-N9, SEQ ID NO:21; HR-N10, SEQ ID NO:23; HR-N11, SEQ ID NO:25; HR-N12, SEQ ID NO:27; HR-N13, SEQ ID NO:29; HR-N14, SEQ ID NO:31; HR-N15, SEQ ID NO:33; HR-N16, SEQ ID NO:35 AND HR-N17, SEQ ID NO:37.

**At page 15, please rewrite Paragraph [0073] as follows:**

[0073] Figure 19 illustrates HR-C peptide sequences, HR-C1 to HR-C4. The nucleotide sequences encoding HR-C1, HR-C2, HR-C3 and HR-C4 are set forth in SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43 and SEQ ID NO:45, respectively. The amino acid sequence of HR-C1(1147-1185) is set forth in SEQ ID NO:66.

**At page 15, please rewrite Paragraph [0074] as follows:**

[0074] Figure 20 illustrates a nucleotide sequence for a SARS HR-N region. See SEQ ID NO:3, or nucleotides 2644-3033 of SEQ ID NO:1.

**At page 15, please rewrite Paragraph [0075] as follows:**

[0075] Figure 21 illustrates a nucleotide sequence encoding SARS S protein  
~~nucleotide sequence~~ (SEQ ID NO:1).

**At page 15, please rewrite Paragraph [0076] as follows:**

[0076] Figure 22 illustrates the native HR-C sequence (SEQ ID NO:48) and analogs of HR-C peptides (SEQ ID NOs:67-74).

**At page 18, please rewrite Paragraph [0087] as follows:**

[0087] When mixed together, the two peptide regions (HR-N and HR-C) associated to form a stable alpha-helical 6-stranded structure (trimer of heterodimers). Systematic peptide mapping showed that a site of interaction between the HR-N and HR-C regions is between residues 916 to 950 of the HR-N domain (See SEQ ID NO:50) and residues 1151 to 1185 of the HR-C domain (see SEQ ID NO: 46). Additionally, interchain disulfide-bridge experiments showed that the relative orientation of the HR-N and HR-C helices in the complex was antiparallel.

**At page 18, please rewrite Paragraph [0089] as follows:**

[0089] Figure 2. (Top); Sequence of the predicted HR-N region from residues 882 to 1011 of the SARS-CoV S protein (SEQ ID NO:4). The a and d positions of the strongest predicted coiled-coil heptad repeats (abcdefg)<sub>n</sub> are shown above the sequence. The a and d positions of the alternate weaker scoring heptad repeat is shown below the sequence. (Middle); The names and sequence regions (denoted in brackets) of the HR-N peptides used in this study. The rectangle denotes the HR-N sequence from residue 882 to 1011 (SEQ ID NO:4). The hashed areas denote regions 1, 2 and 3 (Figure 1). The bars underneath the HR-N domain indicate the locations of the peptides within the HR-N region used in this study. (Bottom); The sequence of the predicted HR-C domain from residues 1147 to 1185 (SEQ ID NO:40). The a and d positions are shown above the sequence. The names, sequences and locations (bars) of the HR-C peptides used in this study are shown below the HR-C domain.

**At page 19, please rewrite Paragraph [0092] as follows:**

[0092] Figure 5. Interaction between HR-N and HR-C peptides. (A) Circular dichroism spectra of HR-N10 peptide alone. Spectra were recorded at 25°C in a 0.1 M KCl, 0.05 M PO<sub>4</sub>, pH 7 buffer. For the spectrum containing 50% trifluoroethanol (TFE), the above buffer was diluted 1:1 (v/v) with TFE. (B) CD spectrum of a 1:1 molar complex between HR-N10 and HR-C1 peptides at ~~25°C~~ 25°C in a 0.1 M KCl, 0.05 M PO<sub>4</sub>, pH 7 buffer. Peptide concentrations were 80 µM. The theoretical spectrum for two non-interacting peptides is shown for comparison. This spectrum is generated by adding the individual peptide spectra at the same concentrations. (C) Temperature denaturation profiles of HR-C1 alone and a 1:1 molar HR-C1 with HR-N10 complex monitored by CD at 222 nm in a 0.1 M KCl, 0.05 M PO<sub>4</sub>, pH 7 buffer. Concentrations were 100 µM. Fraction folded was calculated as described in figure 2 legend.

**At page 21, please rewrite Paragraph [0098] as follows:**

[0098] Plasmid construction. Production of the peptide corresponding to SARS S protein amino acids 882-973 (HR-N1) was done using directional sub-cloning and bacterial expression techniques. PCR fragments were prepared from the plasmid SARS S #18 (~~containing encoding~~ the entire S protein from SARS, Urbani strain (Genbank Accession No. AY278741)). Primers were designed to incorporate an NdeI restriction site upstream (GTACGTACGCATATGATGCAAATGGCATATAG GTTC) (SEQ ID NO:103) and an EcoRI site downstream (GCGAATTCCCTTTGTCGTCGTCGTC GCAGCCGCCTACCTCCGCCTCGACTTTATCAAG) (SEQ ID NO:104) of the amplified fragment. The NdeI site contains an ATG start codon just upstream of the SARS sequence. The reverse primer was engineered to incorporate the amino acid sequence Gly-Gly-Cys-Asp-Asp-Asp-Asp-Lys (SEQ ID NO:107) to insert an enterokinase site (DDDDK) (SEQ ID NO:108) just downstream of the synthesized sequence, and just upstream of the EcoRI site. The nucleotide fragment ~~corresponding encoding~~ to residues 882-973 (SEQ ID NO:6) was amplified by PCR and subcloned into the EcoRI/NdeI site of the pT7SH6 plasmid, in frame with the 6-His tag directly downstream of the EcoRI site to create the plasmid pT7SH6 SARS 882-973. pT7SH6 contains an oligonucleotide-encoded 6-His tag followed by two stop codons cloned into the EcoRI-

BamHI sites of pT7.7 so that cloning into the EcoRI site creates a C-terminal protein fusion encoding NSHHHHHHXX (SEQ ID NO:109).

**At page 22, please rewrite Paragraph [0099] as follows:**

[0099] Plasmid pT7.7 (based on the plasmids of Tabor and Richardson (48)) enables genes to be expressed by T7 RNA polymerase using the highly efficient translation signals of T7 gene 10 by cloning into and NdeI (CATATG) site where the ATG is the initiation codon. Plasmid SARS S #18 was created by cloning the full length SARS S protein coding sequence into pcDNA3.1/V5-His TA cloning vector (Invitrogen, Carlsbad, CA). The SARS S sequence was amplified using reverse-transcription from SARS genomic RNA (W. Bellini, CDC, Atlanta, GA), followed by PCR using primers F21488 (CACCATGTTTATTTTCTTATTATTT) (SEQ ID NO:105) and R25259 STOP (TTATGTGTAATGTAAT TTGACACC) (SEQ ID NO:106). The reverse primer contains a stop codon to halt transcription before the V5-6-His tag in the cloning vector.

**At page 25, please rewrite Paragraph [0107] as follows:**

[0107] To identify the heptad repeat (HR) regions within the SARS-CoV S protein, we utilized the coiled-coil prediction algorithm STABLECOIL (53). Figure 1A shows the graphical output of the analysis using a 35 residue window width. Two coiled-coil regions are predicted; the HR-N coiled-coil (residues 882 to 1011 of SEQ ID NO:2) and the HR-C coiled-coil (residues 1147 to 1185 of SEQ ID NO:2) with an interhelical domain of approximately 140 amino acid residues between HR-N and HR-C. The HR-C region is located adjacent to a predicted transmembrane region (residues 1194-1226 of SEQ ID NO:2), and the HR-N region is located C-terminal to a region of high sequence similarity to the fusion peptide (residues 851-882 of SEQ ID NO:2) (Figure 1B). The coiled-coil prediction analysis also indicates that the HR-N region can be further sub-divided into three regions (1, 2 and 3) based on the assignment of the heptad a and d positions (data not shown). The region where the heptad registers change is denoted as a frameshift/hinge region (Figure 1B). Also predicted is a second (lower scoring) heptad repeat register within HR-N region 2 which can run continuous with the heptad register

observed in HR-N region 3 (shown below the sequence in Figure 2, top). The presence of this alternate heptad register indicates that hydrophobic residues also occur in the interfacial e and g positions in this location. In contrast, the HR-C coiled-coil region shows only a single continuous heptad register (Figure 2, bottom).

**At page 26, please rewrite Paragraph [0109] as follows:**

[0109] To examine the structural characteristics of the HR-N and HR-C regions of the SARS CoV S protein, peptides (see Figure 2) corresponding to various regions between residues 882 and 1185 of SEQ ID NO:2 were synthesized and analyzed by circular dichroism (CD) spectroscopy. Figure 3 (panels A and B) show representative far UV CD spectra and thermal denaturation profiles of the HR-N1, HR-N3, HR-N4, HR-N5 and HR-C1 peptides. Analysis of the HR-N1 peptide, which represents almost the entire length of the predicted HR-N region showed limited solubility in 0.1 M KCl, 0.05 M PO<sub>4</sub>, pH 7 buffer, thus the CD study could only be carried out at 15 μM. The CD profile of HR-N1 showed the characteristic α-helical spectrum with double minima at 208 and 222 nm. The molar ellipticity at 222 nm (-15,100°C) corresponds to 40 helical residues out of the maximum 62 helical residues induced in 50% TFE for the molecule under the conditions analyzed. Temperature denaturation of HR-N1 showed the α-helical structure had high stability with 50% of its structure unfolded at 77°C (Table 1).

**At page 45, please rewrite Table 3 as follows:**

Table 3. Sub-peptides of HR-N10 and HR-C4 based on heptad units.

Amino acids of S protein (with reference to SEQ ID NO:2)

Peptide name	Start	End	Length	Sequence
<b>HR-N10</b>	<b>916</b>	<b>950</b>	<b>35</b>	<b>IQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSS</b>
Heptad 1	916	922		IQESLTT
Heptad 2	923	929		TSTALGK
Heptad 3	930	936		LQDVVNQ
Heptad 4	937	943		NAQALNT
Heptad 5	944	950		LVKQLSS
2 heptad units				
1,2	1151	1164	14	IQESLTTTSTALGK
2,3	923	936	14	TSTALGKLQDVVNQ
3,4	930	943	14	LQDVVNQNAQALNT
4,5	937	950	14	NAQALNTLVKQLSS
3 heptad units				
1,2,3	1151	1171	21	IQESLTTTSTALGKLQDVVNQ
2,3,4	923	943	21	TSTALGKLQDVVNQNAQALNT
3,4,5	930	950	21	LQDVVNQNAQALNTLVKQLSS
4 heptad units				
1,2,3,4	1151	1178	28	IQESLTTTSTALGKLQDVVNQNAQALNT
2,3,4,5	923	950	28	TSTALGKLQDVVNQNAQALNTLVKQLSS

<b>HR-C4</b>	<b>1151</b>	<b>1185</b>	<b>35</b>	<b>ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL</b>
Heptad 1	1151	1157		ISGINAS
Heptad 2	1158	1164		VVNIQKE
Heptad 3	1165	1171		IDRLNEV
Heptad 4	1172	1178		AKNLNES
Heptad 5	1179	1185		LIDLQEL
2 heptad units				
1,2	1151	1164	14	ISGINASVVNIQKE
2,3	1158	1171	14	VVNIQKEIDRLNEV
3,4	1165	1178	14	IDRLNEVAKNLNES
4,5	1172	1185	14	AKNLNESLIDLQEL
3 heptad units				
1,2,3	1151	1171	21	ISGINASVVNIQKEIDRLNEV
2,3,4	1158	1178	21	VVNIQKEIDRLNEVAKNLNES
3,4,5	1165	1185	21	IDRLNEVAKNLNESLIDLQEL
4 heptad units				
1,2,3,4	1151	1178	28	ISGINASVVNIQKEIDRLNEVAKNLNES
2,3,4,5	1158	1185	28	VVNIQKEIDRLNEVAKNLNESLIDLQEL

Appl. No. 10/597,914  
Amendment dated October 28, 2009  
Reply to Office action mailed April 29, 2009

**Amendments to the Sequence Listing:**

A replacement Sequence Listing is provided herewith. Applicants request that it be entered and replace any prior Sequence Listings.